Engineering a hydrogen biosensor: selection of overproducing nitrogenase variants for biohydrogen production

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Biologically-produced hydrogen (H_2) or "biohydrogen" is one promising source of renewable energy. A number of microorganisms are being studied as potential producers of biohydrogen through biophotolysis, indirect biophotolysis, photo-fermentations or dark-fermentations. Microorganisms produce H_2 by the activity of either hydrogenases or nitrogenases: Hydrogenase enzymes catalyze the reaction: $2H^+ + 2e^- \leftrightarrow H_2$ whereas nitrogenases catalyze the reduction N_2 with the following limiting stoichiometry: $N_2 + 8H^+ + 8e^- \leftrightarrow H_2 + 2NH_3$. In this work, we have coordinated aspects of both pathways to develop optimized biocatalysts for hydrogen overproduction using the following steps:

1. Engineering a hydrogen responsive genetic circuit in the purple non-sulphur nitrogen-fixing bacterium *Rhodobacter capsulatus* SB1003: *R. capsulatus* carries nitrogenase and hydrogenase enzymes able to produce H_2. It also carries a system to detect H_2 that is composed of three proteins: a H_2-sensor hydrogenase (HupUV), a histidine kinase (HupT) and a response regulator (NtrC-like transcription factor, HupR) (Vignais *et al*., 2005). In the presence of H_2, this sensor triggers expression of hydrogenase structural and biosynthetic genes. Taking advantage of this system, we have introduced a reporter gene under the control of hupS promoter and removed the uptake hydrogenase, generating a new biological-sensor strain capable of accumulating and detecting the presence of both exogenous H_2 and the H_2 produced by its own nitrogenase. This biotechnological tool allows us to obtain a measurable and proportional signal when H_2 is present in the cell.
2. Generating variants of the molybdenum nitrogenase structural genes *nifH*, *nifD* and *nifK*: we are using in vitro evolution techniques to perform random mutagenesis in these genes with a controlled mutation rate. The resulting variants were cloned under *nifH* promoter control into a broad-host-range vector (Kovach *et al*., 1995) optimized for diazotrophic conditions. Libraries obtained (around 4 x 10^6 clones) were introduced and expressed in the strain carrying the modified biological hydrogen sensor.

The suitable combination of both tools results in the development of a genetic circuit for the high-throughput screening of H_2 overproducing nitrogenase variants thus allowing detection and isolation of clones that present a significant signal increased, through the use of cell-sorting cytometry. Thus far, around 1500 clones have been successfully selected by this method, confirming the possibility of using the designed system to select hydrogen-overproducing enzymes.

References

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